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26 May 2014

Version of attached file:

Published Version

Peer-review status of attached file:

Peer-reviewed

Citation for published item:

Shutt, K. and Heistermann, M. and Kasim, A. and Todd, A. and Kalousova, B. and Profosouva, I. and Petrzelkova, K. and Fuh, T. and Dicky, J.-F. and Bopalanognako, J.-B. and Setchell, J.M. (2014) 'Effects of habituation, research and ecotourism on faecal glucocorticoid metabolites in wild western lowland gorillas : implications for conservation management.', *Biological conservation.*, 172 . pp. 72-79.

Further information on publisher's website:

<http://dx.doi.org/10.1016/j.biocon.2014.02.014>

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Effects of habituation, research and ecotourism on faecal glucocorticoid metabolites in wild western lowland gorillas: Implications for conservation management

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ARTICLE INFO

Article history:

Received 4 March 2013

Received in revised form 3 February 2014

Accepted 11 February 2014

Available online 13 March 2014

Keywords:

Conservation

Ecotourism

Faecal-glucocorticoids

Habituation

Primate

Stress

Wildlife

ABSTRACT

Wildlife tourism is proliferating worldwide and has the potential to raise revenue for conservation as well as public awareness of conservation issues. However, concerns are growing about the potentially negative influence of such tourism on the wildlife involved. We investigate the effects of habituation, ecotourism and research activities on levels of faecal glucocorticoid metabolites (FGCMs), a proxy for physiological stress, in wild western lowland gorillas (*Gorilla gorilla gorilla*) in the Central African Republic. We compare FGCMs in three human-contacted groups with those in unhabituated gorillas. We also explore how human–gorilla contact influences FGCMs of a gorilla group undergoing habituation and investigate how measures of general human–gorilla contact, tourism and human proximity influence FGCMs in recently and long-term habituated groups. Two of the three human-contacted groups had higher levels of FGCMs than unhabituated gorillas. The group undergoing habituation had the highest FGCMs, which increased up to 21 days following contacts, suggesting a cumulative FGCM response, in line with descriptions of a hormonal adaptation response to a chronic intermittent stressor. FGCMs in habituated groups were significantly associated with increasing frequency of violation of the 7 m distance rule by observers and with a medical intervention but not with other measures of human pressure. Our findings provide critical information for the management of this, and other, species whose conservation depends on habituation for ecotourism.

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1. Introduction

Wildlife tourism is one of the fastest growing sectors of the international tourism market (Fennell, 2012). It has been

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advocated as a tool to conserve species and habitats and can accrue educational and socio-developmental benefits as infrastructure builds around tourism activities (Williamson and Macfie, 2010). Today's tourists desire close, personal, wildlife encounters and are particularly attracted to endangered species in remote, fragile habitats (Williamson and Macfie, 2010). However, a growing number of accounts document behavioural and physiological alterations in the species encountered (Tadesse and Kotler, 2012; Treves and Brandon, 2005; Semeniuk et al., 2009; Velando and Munilla, 2011), causing concerns that the costs of tourism to the focal organisms may outweigh the wildlife conservation benefits

(Butynski and Kalina, 1998; Ferber, 2000; Higginbottom et al., 2003).

All animals must cope with unpredictable occurrences, or stressors, in their environments (Cyr and Romero, 2008). A normal vertebrate stress response involves a release of glucocorticoids (GCs) from the adrenal cortex (Selye, 1955), which protects an organism against the effects of acute stress via activation of various behavioural and metabolic processes, and is adaptive in the short-term (Cyr and Romero, 2008; Wingfield and Romero, 2010). Long-term elevation of circulating GC levels is, however, maladaptive, as it is linked to hyperglycaemia, neuronal cell death, and suppression of the immune and reproductive systems (Cyr and Romero, 2008). Moreover, unpredictable, chronic, intermittent stressors, as typified in wildlife tourism contexts, are thought most likely to cause pathology (Boonstra, 2012; Sapolsky, 1992). As extensive research has linked increased GC output to ill-health, GCs in blood or faeces have often been used to monitor individuals and populations in conservation research (Cyr and Romero, 2008; Tarlow and Blumstein, 2007; Wikelski and Cooke, 2006), and are used increasingly to assess the physiological effects of human disturbance and wildlife tourism on the animals concerned (Behie et al., 2010; Creel et al., 2002; Ellenberg et al., 2007; Pineiro et al., 2012; Zwijacz-Kozica et al., 2013).

Like other charismatic mega-fauna, great apes figure highly on wildlife tourism wish-lists (Williamson and Macfie, 2010). Great ape tourism relies on the intentional 'taming', or habituation, of wild animals, which involves exposing the apes to a habituation team, until they become accustomed to daily visits ('contacts'), appearing to pay little attention and showing minimal aggression to humans (MGVP, 2009). In the early stages of habituation apes typically show behavioural indications of an acute stress response (Blom et al., 2004). However, once the apes are behaviourally habituated it is postulated that they no longer perceive the arrival of humans as a threat (Butynski and Kalina, 1998), and thus cease to mount an adrenocortical response. To the best of our knowledge, however, no study has examined the effect of the process of habituation on HPA axis activity in any mammal species.

It is important to test the effects of habituation on the GC response in great apes, and the assumption that habituated apes no longer experience an elevated GC response when visited by humans, as chronically elevated GCs can lead to a reduction in resistance to disease (Cohen et al., 2007). Additionally, the close genetic relationship between humans and other apes renders habituated apes vulnerable to human diseases (Woodford et al., 2002). This is of particular concern, as gorillas appear to be physiologically less resilient to stressful situations compared to other great apes as demonstrated by the low survival rate of gorillas in zoos and sanctuaries (King et al., 2009). Gorilla tourism sites have adopted regulations in an attempt to reduce the negative effects of human contact on habituated gorillas including maintenance of a 7 m distance between humans and gorillas, limiting tourist visits to one hour each per day with a maximum number of people per group, and prohibiting visits to gorillas by people who have visible symptoms of contagious illness. However, these rules are difficult to enforce and often fail, leading experts to suggest that the risks of close-contact tourism may be greater than previously believed (Sandbrook and Semple, 2006). Here, we investigate the impacts of habituation, research and tourism on the GC response of the critically endangered western lowland gorilla (*Gorilla gorilla gorilla*).

Western lowland gorillas range across many of the least developed countries in central and west Africa. The remaining wild population size is estimated to be 95,000 animals and is predicted to decline by 80 % over the next 66 years (IUCN, 2012). Despite long-term efforts to habituate groups at several sites, only four groups can currently be visited by tourists. It can take 4–8 years to habituate western lowland gorillas, unlike mountain gorilla

groups which can typically be habituated within a year. Tracking difficulties also mean that habituated western lowland gorillas must be followed closely from dawn to dusk to sustain daily contact with the group. In addition, the paucity of habituated western lowland gorilla groups also means that they often serve the interests of multiple stakeholders, including researchers, funding donors, film crews and photographers, as well as tourists. These pressures increase the risks of physiological stress in western lowland gorillas compared to other apes involved in tourism.

We studied a gorilla group undergoing habituation, a recently habituated group, a long-term habituated group, and non-human-contacted, unhabituated gorillas, to test the following hypotheses and predictions:

Hypothesis 1. Contact with humans elicits a GC response in gorillas, but habituation reduces this response over time. We predict that:

- (a) Gorillas undergoing habituation and habituated gorillas exposed to ecotourism and research activities will have higher FGCMs than those that are not exposed to human contact at all.
- (b) Gorillas undergoing the process of habituation will have higher FGCMs than habituated gorillas.
- (c) Long-term habituated gorillas will have lower FGCMs than more recently habituated gorillas.

Hypothesis 2. The process of habituation is perceived as a threat by gorillas. Based on patterns of FGCM excretion in captive gorillas (Shutt et al., 2012), we predict that:

- (a) FGCM levels in gorillas undergoing habituation will peak around 48 h after contact(s) with humans.
- (b) FGCM levels in gorillas undergoing habituation will decrease to pre-contact levels after peaking at 48 h post contact with humans, assuming the gorillas are not subjected to other environmental stressors.

Hypothesis 3. Elements of daily contact with humans still elicit a GC response in habituated gorillas. We predict that:

FGCM levels will increase with increasing levels of human–gorilla contact, measured as: amount of daily human–gorilla interaction; amount of close-follow research activities; the total daily number of people in contact with the gorilla group; occurrence of tourism; duration of tourist visits; total number of tourist groups; total daily number of tourists; frequency of humans following gorillas at <25 m; and frequency of humans approaching to less than 7 m (violating the distance regulation).

2. Material and methods

2.1. Study site

We conducted our study at Bai Hokou (33°N 663109, 316187UTM) and Mongambe (33°N 654357, 322606 UTM) study sites, in the Dzanga-Sangha Protected Areas (DSPAs) in the Central African Republic (CAR). The DSPAs are co-managed by the CAR government, the World Wildlife Fund and the Primate Habituation Programme (PHP). For a more detailed description of the study sites see Carroll (1986). Gorilla habituation aimed at developing ecotourism and research activities at Bai Hokou commenced in 1997. Today, tourists (426 in 2011) can visit a long-term habituated group of gorillas (Makumba) at Bai Hokou and another more

recently habituated group (Mayele) at Mongambe. A further gorilla group (Mata) is undergoing the process of habituation at the Bai Hokou site. With the development of tourism the PHP has taken measures to reduce the health risks that close human contact poses to gorillas, such as maintenance of a 7 m distance from gorillas and maximum number of six visitors over two, 1 h visits per day, with a maximum of three tourists per group.

2.2. Study subjects

We collected observational data and faecal samples from the three known gorilla groups and also from non-human-contacted, unhabituated gorillas in the same area between November 2010 and December 2011 (Table 1).

2.3. Observational sampling

For habituated groups, we collected contact data during daily follows (follow data for long-term habituated group $n = 250$, for recently habituated group $n = 116$) and tourist visits (tourism follow data for long-term habituated group $n = 57$, for recently habituated group $n = 16$) when the total number of people did not exceed the maximum permitted group size of six (including two trackers and a guide). Daily contact duration was 6–9 h per day. We took instantaneous scans (Altmann, 1974) on human–gorilla proximity every 10 min, noting the distance of each visible gorilla in meters up to 25 m, then as >25 m, as pilot data suggested that distances >25 m were often obscured by vegetation. We collected all occurrence data on the frequency of humans breaking the 7 m distance rule when approaching a gorilla. We made a further record if the gorilla moved away and the human approached again to <7 m, but did not make a further record if the human remained at less than 7 m. We disregarded occurrences where gorillas approached humans.

For the group undergoing habituation, we recorded the number of days contacts were made (130 contacts on 90 days over the study period) and the number of contacts per day (range 0–3).

2.4. Faecal sampling

We collected a small portion (~ 0.5 g) of fresh faeces (up to 30 min after defecation) when following habituated gorillas. For the gorillas undergoing habituation and unhabituated gorillas we collected samples from the trails if estimated to be less than 30 min old and from fresh (defecated the same day) samples from nest sites. We were only able to observe defecation, and thus identify all samples to the level of the individual for the long-term habituated group. We attempted to equalise sampling effort across groups; however, sampling from unhabituated gorillas was very difficult as teams had to locate dung from wild groups or individuals. Experienced trackers assisted with age/sex class identification of faeces from the trails and nests according to faecal bolus size, nest size and position (Tutin et al., 1995). We followed validated

methods to avoid variation in our FGCM measurements resulting from sampling, extraction and storage (Shutt et al., 2012). We previously detected no effects of urine contamination on FGCMs (Shutt et al., 2012). Nevertheless, we took all sample portions from the centre of the faecal bolus where it should not have been affected by urine.

We shipped the samples to the German Primate Centre Endocrinology Laboratory. We conducted FGCM measurements using a 11β -hydroxyetiocholanolone ($3\alpha,11\beta$ -dihydroxy-CM) enzyme immunoassay which we have previously shown to be physiologically, biologically and immunologically valid for measuring faecal glucocorticoid output in the western lowland gorilla (Shutt et al., 2012). Inter-assay coefficients of variations for these measurements were 9.2 % (high value quality control) and 15.1 % (low value quality control). We removed any samples with known complications (e.g., seeds discovered in the faecal matrix or alcohol evaporation), leaving 1175 samples for analyses.

2.5. Independent variables: human–gorilla contact measures

For habituated groups, we used daily PHP records to establish contact days, and calculated contact duration (humans following the gorillas with or without direct observation), duration of direct visual contact (mutual line of visibility) and the total number of people with the group each day. For each day, we recorded whether researchers followed individual gorillas closely, the presence of medical researchers and film crews or professional photographers, whether tourists visited the gorillas, how many tourist groups visited the gorillas, and the total number of tourists (Table 2). We calculated the mean daily distance to the gorillas, splitting distances <25 m from those >25 m, and calculated the hourly frequency at which humans broke the 7 m distance regulation, correcting for the number of gorillas in the group. We also used PHP project data on daily rainfall (range 0–75.5 ml) and temperature (range 11–89° Fahrenheit) where available (long-term habituated group only).

For the group undergoing habituation, we used the number of human contacts with the group each day as a measure of habituation pressure.

2.6. Dependent variable: FGCM measures

FGCMs decrease over time in unpreserved gorilla faeces (Shutt et al., 2012). The temporal degradation pattern is best described by a polynomial fit, $Y = 0.0039x^2 - 0.0844x + 0.9976$, where x = time between defecation and preservation. We calculated the age of faecal samples collected from nests using the precise collection time and the average time gorillas leave their nests (5:30 am: K. Shutt pers. obs; A. Todd pers. comm) and used this information to compensate for hormone degradation in samples that were not collected immediately. We obtained a corrected value (A) from the original wet hormone content value (B) using $A = B * 100/Y$.

Table 1

Details of study groups, including habituation status, location, group composition, faecal sample identification level and number of faecal samples.

Study groups				
Habituation status	Long-term Habituated	Recently Habituated	Under Habituation	Unhabituated
Year Habituation/Tourism Commenced	2000/2004	2005/2009	2008/N/A	N/A
Group size ^a	9	14	$\sim 8/9$	N/A
Faecal sample identification	Individual	Individual & Age Category	Age Category	Age Category
Total faecal samples used in analyses	554	250	301	70

Data are adjusted accordingly.

^a Group size decreased from 10 to 9 during the study period in the long-term habituated group due to female off-spring immigration and increased from 13 to 14 with the birth of a new infant at the end of the study period in the recently habituated group.

Table 2

Details of observational data collected to establish measures of human–gorilla contact for both the long-term habituated group and the recently habituated group.

Observation	Long-term habituated group	Long-term habituated group
Overall contact duration range (mins)	45–607	202–632
Direct visual contact range (mins)	45–598	202–615
Total daily number of people with the group range	1–19	N/A
Close-follow research days	48	N/A
Medical intervention days	9	35
Film/Camera crew days	41	N/A
Tourism days	57	16
Tourism groups range	1–4	1–3
Total daily number of tourists range	1–11	1–11

We found no diurnal variation in FGCMs (Shutt et al., 2012), so used all samples in analysis. We express all hormone data as hormone per faecal wet mass. We applied a 48 h time lag when fitting observational data to the hormone data as western lowland gorilla FGCMs peak 48 h after a stressor (Shutt et al., 2012). We used the natural logarithm of our hormone data (lnFGCM) in all analyses to achieve a normal distribution.

2.7. Statistical analysis

Before testing our predictions, we ran a series of analyses using generalised linear mixed effect models (GLMM) with a Gaussian distribution and identity link to investigate variation in FGCMs between age-class categories, the two sexes, and wet (April–October) and dry months (November–March). We also explored the relationship between FGCMs and daily rainfall and mean temperature.

2.8. Hypothesis 1

To test the predictions of hypothesis 1, we used a GLMM to compare FGCMs of the three human-exposed groups (the long-term habituated group, the recently habituated group and the group undergoing habituation) with those of unhabituated gorillas, and to one another. We used age category as a random effect as the data were not always uniquely identified to individual gorillas in the recently habituated gorilla group, the group undergoing habituation, and the unhabituated gorillas. We then applied adjustments for multiplicity corrections based on a false discovery rate (Benjamini and Hochberg, 1995).

2.9. Hypothesis 2

To test the predictions of hypothesis 2, we used FGCM data from the group undergoing habituation. We used GLMMs with age category as a random effect as the data for this group were not uniquely identified to individual gorillas. To test prediction 2a we compared FGCMs in samples collected up to 48 h after contacts were made with the group (range 1–3 contacts on one day) with those before contacts were made, as FGCMs peak ~48 h after exposure to a stressor (Shutt et al., 2012). To test prediction 2b, we tested for an association between FGCMs and the number of days since the last contact(s) was made, including samples collected after 48 h.

2.10. Hypothesis 3

We used GLMM to test the predictions of hypothesis 3, setting gorilla individual as a random effect for data from the long-term habituated group. We used multiple linear regression models (assuming all observations were independent) for analyses of the data from the recently habituated group. After testing all the

variables we excluded those with non-significant bivariate associations with FGCMs at $p > 0.25$. We then performed separate analyses for three categories of the remaining variables: general human–gorilla contact; tourism-specific contact; and human–gorilla proximity. We treated the three categories separately as there were substantial differences in the amount of data available for each category. For the long-term habituated group there were 510 observations for the general human–gorilla contact, 141 for tourism-specific contact, and 334 for human–gorilla proximity. For the recently habituated group, there were 205 observations for the general human–gorilla contact, 31 for tourism-specific contact, and 96 for human–gorilla proximity. We applied adjustments for multiplicity correction for the final models based on false discovery rates (Benjamini and Hochberg, 1995).

In building our models, we first tested for associations between FGCMs and the occurrence of tourism (yes/no), the duration of daily human–gorilla contacts (min), duration of daily direct visual observation (mins) and the total daily number of people with the group. We also tested for relationships between FGCMs and close-follow research, a medical intervention, and film crew/photographer presence (all yes/no) in the same model. Second, we used tourism-specific data and tested for associations between FGCMs and the duration of tourism visits (mins), the total daily number of tourist groups and the total daily number of tourists. Finally, we tested relationships between FGCMs and human–gorilla proximity when gorillas were at <25 m and >25 m, and the frequency of violation of the 7 m distance regulation by humans (long-term habituated group range 0.19–10.5/h, mean = 1.83/h; recently habituated group 0.29–3/h, mean = 1.29/h). We could not run all tests for the recently habituated group, as sampling difficulties greatly reduced the data set.

We conducted all statistical analyses in R2.14.2. We report the mean difference (MD), the standard error (SE) and the p -value for each association tested and the slope (S), its standard error (SE) and p -value for quantitative predictors. We show the variance (Var) and standard deviation (SD) for the random effects component of the GLMM models in the results tables.

3. Results

We found no significant relationships between mean FGCMs and age-class, sex, season, and mean daily temperature or rainfall in any of the gorilla groups where data were available to test (GLMM: all $p > 0.25$, data not shown). We, therefore, excluded these variables from further analyses.

3.1. Hypothesis 1

In line with prediction 1a, both the group undergoing habituation and the recently habituated group had significantly higher FGCMs than unhabituated gorillas (GLMM: MD = 0.23, SE = 0.07,

$p = 0.01$; MD = 0.018, SE = 0.087, $p = 0.01$). The comparison between the FGCMs of the long-term habituated group and unhabituated gorillas gave a p -value of 0.05 (GLMM: MD = 0.013, SE = 0.07, $p = 0.05$). In line with prediction 1b, FGCMs in the group undergoing habituation were also significantly higher than those in the long-term habituated group (GLMM: MD = 0.01, SE = 0.04, $p = 0.01$). However, contrary to prediction 1c, FGCMs in the recently habituated group were not significantly different to those in the long-term habituated group (GLMM: MD = 0.05, SE = 0.04, $p = 0.27$, Fig. 1, Table A1).

3.2. Hypothesis 2

We found some support for prediction 2a, that FGCM levels in gorillas undergoing habituation will peak 48 h after a contact with humans. FGCMs were significantly higher than pre-contact levels 48 h after 3 contacts with humans in the group undergoing habituation (GLMM: MD = 0.350, SE = 0.135, $P = 0.010$), although they were not significantly different from pre-contact levels within the predicted 48 h period after only 1 or 2 contacts with humans (1 contact GLMM: MD = 0.048, SE = 0.085, $p = 0.572$; 2 contacts: MD = 0.234, SE = 0.209, $p = 0.265$). Contrary to prediction 2b, that FGCMs would decrease to pre-contact levels after 48 h, they continued to rise, and rose significantly for up to 21 days after contact(s) (GLMM: MD = 0.020, SE = 0.008, $p = 0.011$, Fig. 2, Table A2).

3.3. Hypothesis 3

The medical intervention period was significantly associated with increased FGCMs for both the long-term habituated group and the recently habituated group (GLMM: MD = 0.36, SE = 0.11, $p = 0.01$; MD = 0.21, SE = 0.08, $p = 0.01$). An increasing frequency of humans breaking the 7 m distance regulation was also significantly associated with higher FGCMs in both habituated groups, but this relationship only remained significant in the recently habituated group after multiplicity correction (GLMM: MD = 0.015, SE = 0.05, $p = 0.01$). We found no significant difference in FGCM levels relating to the effects of tourism days or other types of contacts, nor in our detailed analysis of tourism variables (Table A3).

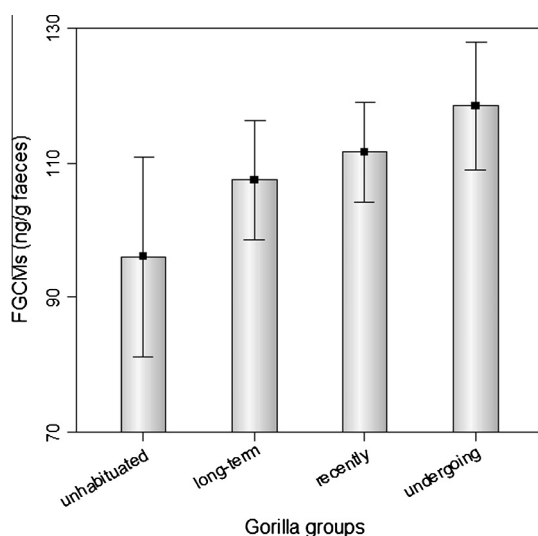


Fig. 1. Mean \pm SD FGCM values for gorillas that are unhabituated, long-term habituated, recently habituated and undergoing habituation with 95% confidence intervals.

4. Discussion

We found that two, (and possibly all three) of the human-contacted gorilla groups that we studied had higher mean FGCMs than unhabituated gorillas. This supports both a physiological habituation effect in gorillas, as well as an ongoing influence of human contact on gorilla physiology, despite habituation. The group undergoing habituation had the highest FGCMs, which increased up to 21 days after contacts. This response may represent an anticipatory FGCM response to a chronic intermittent stressor. FGCMs in habituated groups were significantly associated with increasing frequency of violation of the 7 m distance rule by observers and with a medical intervention, but not with other measures of human pressure. This suggests that current human–gorilla contact regulations at the study site are adequate, but that general human–gorilla proximity regulations require revision. Our findings emphasise the importance of conducting habituation processes quickly and efficiently, and of monitoring accepted human–wildlife management regulations to protect gorillas and other human-exposed animal species.

4.1. Hypothesis 1: contact with humans elicits a GC response in gorillas, but habituation reduces this response over time

We found evidence to support this hypothesis, as gorillas undergoing habituation and recently habituated gorillas had significantly higher FGCMs than un-habituated gorillas. These findings are similar to those for other species: tourism-exposed black howler monkey groups (*Alouatta pigra*), European pine martins (*Martes martes*) and little penguins (*Eudyptula minor*) all had significantly higher faecal cortisol than non-tourism-exposed groups of the same species (Behie et al., 2010; Barja et al., 2007; Turner, 2001). However, although the mean FGCM values of the long-term habituated group were higher than those of the unhabituated gorillas, as illustrated visually in Fig. 1, the difference was statistically ambiguous ($p = 0.05$). An interpretation based on a lack of an effect would suggest that long-term habituation reduces the GC response to human contact to a similar level to that when there is no contact at all. However, given that the data relate to physiological alterations in a critically endangered animal resulting from accepted management practices, it is important to recognise the possibility that the differences may represent an ongoing effect of human–gorilla contact despite habituation, which would be in support of prediction 3.

The FGCM levels of the group undergoing habituation were significantly higher than those of the long-term habituated group and unhabituated gorillas, which supports prediction 1b, that gorillas undergoing the process of habituation will have higher FGCMs than habituated gorillas, although FGCM levels were not higher than the recently habituated group. These results are similar to a study that showed that FGCMs in unhabituated orangutans that had been followed by humans for several days were higher than those of habituated animals after human contacts (Muehlenbein et al., 2012). Together, these two studies provide evidence that unhabituated apes mount a stronger GC response to human contact than habituated apes.

The most parsimonious explanation of the differences in FGCMs we observed between the gorillas groups relates to their habituation status. That is, for gorillas, not being contacted by humans at all may be the least stressful situation compared to being under habituation or recently habituated. However, with time, habituation may result in gorillas perceiving human contact as less of a threat, and therefore less stressful, so that long-term habituated animals have lower basal FGCM levels than those undergoing habituation. However, these between-group comparisons should

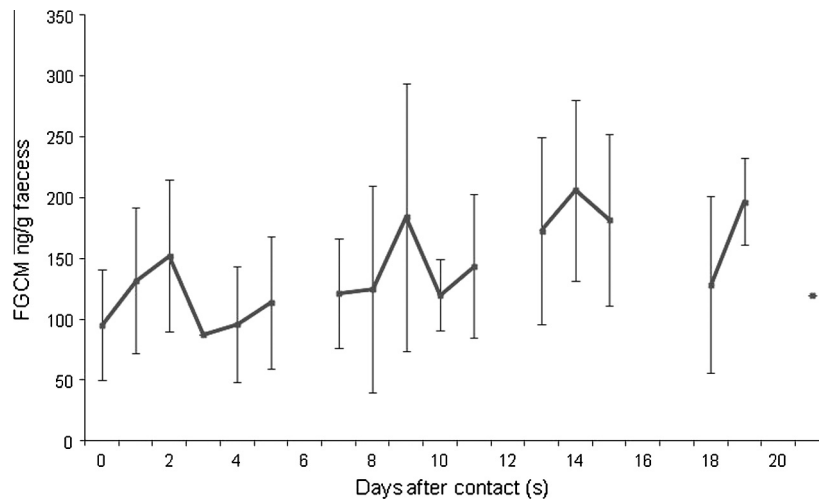


Fig. 2. Relationship between mean \pm SD FGCm values and the number of days after humans made contact(s) with the group undergoing habituation. 'Day 0' represents day of contact (therefore the peak FGCm response to contact would be expected 48 h later).

be interpreted with caution, and do not necessarily allow us to conclude that the variation in FGCm levels are a direct result of human-exposure (Breuner et al., 2013). For example, differences in FGCms may simply reflect a normal response to stimuli, which does not necessarily equate to fitness costs (Treves and Brandon, 2005) and individuals may have different basal levels of stress hormones (Ostner et al., 2008).

Environmental stressors are not equal across individuals or groups, and FGCm differences may be related to seasonal, diet, and life history differences (Romero, 2002) as well as behavioural differences (Muehlenbein et al., 2012). We found no significant individual or seasonal effects on FGCms, and controlled for age-sex class in our analyses, making these unlikely explanations for the between-group variation in FGCms. However, we were unable to control for nutritional differences between groups, or the effects of any demographic changes in gorillas undergoing habituation and unhabituated gorillas. Furthermore, GC responses to human exposure may be influenced by animal temperament and context (Martin and Réale, 2008). Therefore, we adopted a stronger, more informative approach and explored a gorilla groups' FGCm response to habituation (hypothesis 2), and the response of habituated groups to measures of daily human contact (hypothesis 3).

4.2. Hypothesis 2: the process of habituation is perceived as a threat by gorillas

Although our specific predictions that FGCms would significantly peak at 48 h and decrease thereafter were not fully supported, our findings still provide support for this hypothesis. We observed a significant peak (approximately 50% elevation) in FGCms 48 h after three contacts (but not one or two contacts) had been made with the group on one day. This result is similar to the results of an experimental study which found that sparrows exposed to three stressors per day had significantly increased endogenous corticosterone levels compared to those that received only one (Busch et al., 2008). This may suggest that the disturbance caused by one or two contacts per day is not perceived as a great enough stressor by the gorillas at this stage in their habituation to cause significant alterations to the FGCm response, but that the disturbance caused by three contacts per day is. We also detected a significant increase in FGCms after the initial peak response within the 48 h period: FGCms continued to rise over time between contacts for up to 21 days, instead of decreasing after 48 h as we predicted. This finding suggests that human con-

tact (irrespective of the number of contacts per day) is perceived as a disturbance, which results in an increased GC release by the gorillas during the process of habituation. This finding contrasts with the FGCm response to human-visitation in habituated and unhabituated orang-utans, which returned to baseline levels within 48 h (Muehlenbein et al., 2012). As it is not possible to carry out physiological validation using sedation or ACTH challenge with the wild gorillas, we cannot conclude whether the elevated FGCm levels rose sufficiently above their basal levels to become bound to the hormone receptor that activates the stress response (Breuner et al., 2013) and thus represent a true stress reaction. However, if allostatic overload persists and GCs are above basal levels for days or weeks, this may result in what is often termed "chronic stress", which can alter baseline GCs, stress-level GCs and/or the duration of the GC response to stressors (Busch and Hayward, 2009).

Following the peak in FGCm levels on day 2 after contacts, we also observed a drop on days 3, 4 and 5, followed by a more pronounced and sustained rise with a second, similarly-sized peak on day 14. The cause of the second peak is largely open to interpretation. It may be that this response is due to a state of anticipatory vigilance (Arthur, 1987; Busch and Hayward, 2009). A single predator attack, or a severe attack by a dominant conspecific, may cause an animal to anticipate more of the same and thus to become chronically stressed (Boonstra, 2012). This response is well-illustrated in humans and other animals responding to chronic unpredictable stressors (Burchfield, 1979; Clinchy et al., 2010; Davis and Levine, 1982), and may have similar cognitive underpinnings to those preceding post-traumatic-stress-disease in humans (Yehuda, 2002; Boonstra, 2012). If the rising FGCm response of the group undergoing habituation is due to a physiological reaction to anticipation of a chronic intermittent stressor (such as habituation), this would offer an explanation as to why the FGCms of unhabituated human-contacted orangutans returned to baseline 48 h later (Muehlenbein et al., 2012), as the orangutans were not undergoing habituation. Alternatively, the sustained rise and second FGCm peak may be indicative of other influential environmental or social stressors in the lives of this gorilla group which we were unable to control for in the study. If this is the case, then our results may suggest that the effect of the habituation process in an FGCm context is no different to other such energetically challenging events. If, however, the second peak illustrates the gorillas physiological response to contacts as a long-term (<21 days) elevation in FGCms, as Fig. 2 suggests, then this may also explain the large variation in FGCm levels observed in the day 0 samples before contacts were

made which we were also unable to control for in the study. Thirdly, like brown bears during the hunting season (Ordiz et al., 2012), gorillas under habituation detect humans before contact is made and adjust their movements accordingly (Blom et al., 2004; Cipolletta, 2003). If the gorillas under habituation perceive a threat of human contact continually, this may combine with the effect that increased physical activity as a result of human avoidance may have on GC release (Li et al., 2012) and contribute to the sustained increase in FGCM levels observed in the days after contacts made with the group undergoing habituation.

4.3. Hypothesis 3: elements of human–gorilla contact may still elicit a GC response in habituated gorillas

We found no associations between FGCMs in the habituated groups and the time humans spent in visual or non-visual contact, the total number of people visiting the group each day, or the occurrence of tourism. These results suggest that current human–gorilla contact regulations are effective at preventing significant FGCM increases. However, variation in the tourism variables was generally small and our methods may not be sufficiently sensitive to detect adrenocortical responses to relatively subtle variation.

Our findings do not, however, necessarily suggest that the long-term and recently habituated gorillas are not chronically stressed, as physiological alterations can occur in response to repeat stressors in the absence of detectable FGCM alterations as a result of physiological response down-regulation, blunting or desensitisation (Busch and Hayward, 2009; Rich and Romero, 2005). This seems to be the case in Magellanic penguins (*Spheniscus magellanicus*) exposed to tourism (Walker et al., 2005) and is well documented in research investigating the stress impacts of wildlife tourism on other animal species (Fowler, 1999; Millspaugh and Washburn, 2004; Müllner et al., 2004).

Our finding of significantly increased FGCMs in both habituated gorilla groups in response to the medical intervention is not surprising as the intervention required close and persistent follows of individual gorillas. We expected this process to elicit a temporary GC response, compensated by a long-term health benefit (details in Walsh et al. in prep). Our most important finding, however, was that transgressions of the 7 m distance rule were associated with an increase in FGCMs in both habituated gorilla groups in initial analyses, and remained significant in the recently habituated group overall, which provides support for prediction 3. Similar findings are reported in tourism-exposed Barbary macaques (*Macaca sylvanus*), where a rise in FGCMs was detected in response to aggressive (possibly closer) interactions with tourists (Maréchal et al., 2011).

4.4. Management implications

Although there is debate as to whether naturally occurring chronic stress results in pathology (Boonstra, 2012), immunosuppression and illness are commonly linked to chronic intermittent stress (Cohen et al., 2007). Given that the group undergoing habituation, the recently habituated group and potentially also the long-term habituated group had higher FGCMs than unhabituated gorillas, it would seem prudent to take all measures possible to reduce potential causes of FGCM elevation in habituated groups and those undergoing habituation in order to reduce potential physiological impacts of their imposed contact with humans.

4.4.1. Habituation

Our findings are important in understanding hormonal habituation and provide a useful tool with which to assess GC variation during the vulnerable phase of habituation. A key implication of

our study is that contacts made with gorillas undergoing habituation elicit a significant FGCM response, which accumulates in the days following contacts, and may be indicative of a chronic stress response. This enforces the need to conduct routine, direct, visual monitoring of gorillas undergoing habituation for indications of ill health such as weight loss, ectoparasitic infection and behavioural alterations. Samples should also be collected non-invasively to monitor health or establish other measures of 'downstream' physiology (Breuner et al., 2013). Furthermore, if the FGCM response to human visitation reduces with increasing habituation as our between-groups comparison suggests, then it would be prudent to carry out habituation efficiently and quickly. This means that the decision to start habituation should not be made without ensuring the availability of funds and mechanisms to locate and monitor gorillas and priority should be placed on completing habituation over research and tourism demands.

4.4.2. Research and tourism with Habituated Groups

We observed that humans often broke the 7 m regulation and that this was linked to increasing FGCM levels in recently habituated gorillas. This suggests that managers should consider increasing the minimum viewing distance beyond 7 m. Research on habituated western lowland gorillas found that behavioural alterations, such as increased visual monitoring of humans and low-level aggression directed at humans, decreased when visitors remained at 10 m from gorillas (Klailova et al., 2010), suggesting that FGCM levels may also decrease, although further research is necessary to test this possibility. A greater distance regulation would also reduce the risks of direct human–gorilla disease transmission and bring the site in line with the most recent IUCN recommendations (Williamson and Macfie, 2010).

Finally, our results may be representative of the effects of human-visitation on other species in similar contexts where habituation for close-contact research and tourism is used for conservation. The implications of our study are likely to be more widely applicable to other such ecotourism and conservation management contexts. The development and application of similar studies to monitor and advise ecotourism management strategies is vital if it is to offer a sustainable wildlife conservation solution.

Acknowledgements

We thank the World Wildlife Fund and the Administration of Dzanga-Sangha Protected Areas in the Central African Republic for assistance with obtaining permits and for collaborative research support, and the Ministre de l'Education Nationale, de l'Alphabetisation, de l'Enseignement Supérieur, et de la Recherche for granting research permission and sample transport permits. Special thanks to all the staff of the Primate Habituation Program for logistical support and assistance, and especially the BaAka trackers in the field. We thank P. Kiesel and A. Heistermann for expert help with hormone analysis, and P. Walsh for information and comments on the manuscript. Funding for this study was generously provided by a NERC/ESRC interdisciplinary PhD studentship, the Primate Society of Great Britain, the International Primatological Society, the Bio-Social Society UK, and Rufford Small Grants for Nature Conservation. We adhered to the research protocols defined by the Administration of Dzanga-Sangha Protected Areas and all research was approved by the Durham University Life Sciences Ethical Review Process Committee. We also thank the anonymous reviewers for their constructive advice on earlier versions of this manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2014.02.014>.

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